

AN ANNOTATED LIST OF MICROFUNGI ISOLATED FROM THE SOILS AROUND PRETORIA, TRANSVAAL

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ABSTRACT

An ecological survey of soil fungi of an alkaline soil from an open-savannah in Transvaal was conducted over a period of one year. During this investigation numerous fungi were isolated by means of various isolation techniques. Descriptions of three of these species are given. Many of the fungi are new records for South African soil. An annotated list of all identified fungi is given in this report.

UITTREKSEL

'N LYS MET AANTEKENINGE VAN MIKROFUNGI GEÏSOLEER UIT DIE GRONDE OM PRETORIA, TRANSVAAL

'n Ekologiese opname van grondfungi van 'n alkaliese grond van die oop-savanna van Transvaal is uitgevoer oor 'n periode van een jaar. Tydens hierdie ondersoek is talryke fungi geïsoleer deur gebruik te maak van verskillende isolasietegnieke. Beskrywings van drie van hierdie spesies word gegee. Baie van die fungi is nuwe rekords vir Suid-Afrikaanse grond. 'n Geannoteerde lys van al die geïdentifiseerde fungi word in hierdie verslag gegee.

INTRODUCTION

Apart from the work of Papendorf and Von Arx (1966), Papendorf (1967, 1969), Papendorf and Du Toit (1967) and Eicker (1969, 1970 a & b) little is known about the microfungal populations of the local soils of natural communities. During an ecological survey of soil fungi of an open-savannah site in the Pretoria district of the Transvaal many fungi were isolated.

MATERIAL AND METHODS

The soil of the sampling area

The sampling site is situated in an undisturbed piece of Sourish Open-Savannah Veld (Acocks, 1953) with the dominant trees being *Celtis africana* Burm. f.; *Acacia caffra* (Thunb.) Willd. and *Diospyros lycioides* Desf. It occurs in the municipal area of Pretoria (25° 28° CA Pretoria) and receives an annual rainfall of 762,5 mm with the maximum precipitation during summer from November to February. The Daspoort Quartsite of the Pretoria Series yields a loam soil with a pH of 7,2.

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Isolation techniques

Soil samples were collected at monthly intervals over a year commencing in May 1971. A sample of about 1 kg, comprising at least ten subsamples, was taken over an area of approximately 10m² using a sterile metal trowel. Samples were analysed within 24h of collection.

For the isolation of fungi three cultural methods were employed: the dilution plate method (Waksman & Fred, 1922) as modified by Menzies (1957), the soil plate method (Warcup, 1950) and a soil washing technique devised by Gams and Domsch (1967). For dilution plates Czapek-Dox-agar with 0.5% Difco yeast extract, rose bengal, 30 µg/ml streptomycin and 5 µg/ml aureomycin was used. The medium used both in the preparation of soil plates and in the washing technique was Peptone-dextrose agar plus rose bengal and the usual antibacterial agents (Martin, 1950). Isolation plates were incubated at 25° C and were periodically examined for at least three weeks in order to allow slow growing species to develop. Subcultures were grown on potato-dextrose or potato-carrot agar. Where growth on these media was unsatisfactory, 2% malt-extract agar or natural substrates such as sterilized grass debris were used. *Penicillium* and *Aspergillus* species were grown on Czapek-Dox-agar, while sterile mycelia were incubated under near-ultraviolet radiation (Leach, 1962) in an attempt to induce sporulation.

In the following list the numbers in brackets indicate the total number of times the fungus was isolated during the year of investigation; asterisks indicate that the species is a new record from South African soil; subcultures of fungi marked + have been deposited at the Commonwealth Mycological Institute, Kew.

OOMYCETES

Saprolegnia UP 812 (1). The only representative of the class. No special techniques, such as baiting, were employed for the isolation of the Saprolegniales.

ZYGOMYCETES

+ *Absidia cylindrospora* Hagem (12). Although the species was isolated on several occasions it was not as common as in Zululand forest soils (Eicker, 1969).

+ *Absidia spinosa* Lendner (1).

Absidia UP 613 (1).

Actinomucor elegans (Eidam) Benjamin & Hesseltine (2)*.

+ *Cunninghamella bainieri* Naumov (1)*.

+ *Cunninghamella echinulata* (Thaxt) Thaxt ex Blakeslee (20). Occurred frequently, especially during autumn (April and May).

+ *Congronelle butleri* (Lendn.) Peyronel & Del Vasco (3).

Mortierella UP 804 (1). I have commented previously on the relative scarcity of species of *Mortierella* in South African soils particularly in terms of the high percentage of occurrence of these fungi in European soils (Eicker, 1969).

+ *Mucor fragilis* Bainier (1)*.

+ *Mucor spinosus* van Tieghem (31)*. This was the most common example of the Zygomycetes. It was isolated in all samples throughout the study.

+ *Mucor racemosus* Fres (6)*.

+ *Mucor* UP 444 (1).

Mucor UP 683 (1).

- +*Zygorhynchus moelleri* Vuill. (21). This homothallic species was characterized by the production of numerous zygospores in culture.
Sterile phycomycete UP 703 (1). Characterized by the production of robust, coenocytic hyphae.

ASCOMYCETES

The asocarp stages of *Penicillium* and *Aspergillus* have been classified with the rest of the species of these genera in the Deuteromycetes.

- +*Achaetomiella irregulare* D. Hawksw. ined (1)*. This new species also represents a first record of the genus for the African continent.
+*Auxarthron umbrinum* (Boudier) Orr & Plunkett (4). See the taxonomic notes below.
+*Chaetomium globosum* (Kunze) Fr. (40). This perithecial fungus was the most common ascomycete, occurring regularly on isolation plates.
+*Chaetomium globosum* var. *flavo-viride* Novak (2).
Chaetomium lucitanicum Gomes (1)*.
+*Chaetomium olivaceum* Cooke & Ellis (1).
+*Chaetomium spinosum* Chivers (14). Isolated only during the dry, warm period (December and January).
+*Chaetomidium subfimetii* Seth (1). See the taxonomic notes below.
Cochliobolus spicifer Nelson (1).
+*Nectria invectra* Pethybr. (1).
Neocosmospora vasinfecta E. F. Smith (3).
Neurospora sitophila Shear & Dodge (2).
+*Pleospora infectoria* Funckel (8). Only the *Alternaria* state of this species developed in culture.
Thielavia terricola (Gilman & Abbot) Emmons (1).

BASIDIOMYCETES

Corticium (Rhizoctonia) solani Kühn (1). Although the basidiocarps of various Basidiomycetes were observed, especially from November to February, no special attempt was made to isolate these fungi from the soil.

DEUTEROMYCETES

Sphaeropsidales

- +*Coniella diplodiella* (Speg.) Petrak & Sydow (7)*. This was the only pycnidial species which occurred with any noteworthy frequency.
+*Coniothyrium fuckelii* Sacc (1).
Phoma UP 502 (1).
Phoma UP 819 (2).
Stagonospora UP 701 (1).
+Unidentified *Sphaeropsidales* UP 790 (1).
Unidentified *Sphaeropsidales* UP 822 (3).

Melanconiales

- Pestalotia* UP 313 (1).
Pestalotia UP 797 (1).

Moniliales *Moniliaceae*

- +*Aspergillus aculeatus* Iizuka (2)*.
+*Aspergillus carneus* (Van Tiegh.) Blochwitz (12)*. Isolated mostly during the summer (October – February).
+*Aspergillus flaviceps* (Bain & Sart.) Thom & Church (1).
+*Aspergillus flavus* (Link) Fr. (8).
+*Aspergillus fumigatus* Fres. (10). Even though some of the isolation plates were regularly incubated at a higher temperature (45° C), *A. fumigatus* was the only thermophilic fungus isolated in this investigation.
+*Aspergillus nidulans* (Eidam) Wint (2).
+*Aspergillus niger* van Tiegh. (4).
+*Aspergillus ochraceus* Wilhelm (1).
+*Aspergillus rugulosus* Thom & Raper (54). This cleistothecial species produced prominent, dark brown Hülle cells and was very commonly isolated throughout the period of investigation.
+*Aspergillus sydowii* (Vuill.) Tiraboschi (1).

- + *Aspergillus ustus* (Bain.) Thom & Church (1).
- + *Aspergillus versicolor* (Vuill.) Tiraboschi (9).
- + *Aspergillus wentii* Wehmer (1)*.
- + *Aspergillus* UP 410 (1).
- + *Aspergillus* UP 505 (1).
- + *Aspergillus* UP 708 (1).
- + *Beauveria bassiana* (Bals.) Vuill. (2).
- + *Gliocladium roseum* Bain. agg. (56). Isolated regularly throughout the investigation.
- + *Gliocladium* cf. *roseum* Bain. agg. (1).
- + *Gliocladium* UP 629 (1).
- + *Monilia* UP 727 (1).
- + *Paecilomyces* UP 450 (28). Isolated periodically during the period of investigation.
- + *Paecilomyces* UP 777 (7).
- + *Paecilomyces* UP 797 (1).
- + *Paecilomyces* UP 820 (1).
- + *Penicillium brevicompactum* Dierckx (1).
- + *Penicillium canescens* Sopp (2).
- + *Penicillium chrysogenum* Thom (8)*.
- + *Penicillium crustosum* (Thom) (1)*.
- + *Penicillium cyclopium* Westling (21). Common during early spring (August and September).
- + *Penicillium decumbens* Thom (1)*.
- + *Penicillium frequentans* Westling (24).
- + *Penicillium herqui* Bain. & Sart. (2).
- + *Penicillium lilacinum* Thom (1).
- + *Penicillium multicolor* G.M.-P. (58). This species was the most common and abundant fungus found in the alkaline soil under investigation. It was evidently not influenced by seasonal changes as it occurred on isolation plates of all soil samples throughout the year of investigation.
- + *Penicillium rainstrickii* Smith (10).
- + *Penicillium thomii* Maire (1)*.
- + *Penicillium waksmanii* Zal (7).
- + *Penicillium* UP 458 (1).
- + *Penicillium* UP 465 (1).
- + *Penicillium* UP 770 (2). This cleistothecial species showed very strong antagonistic properties on the isolation plates. Antibiotics produced by its colonies suppressed the growth of other isolates in its immediate vicinity very markedly.
- + *Penicillium* UP 793 (1).
- + *Scopulariopsis brevicaulis* (Bain.) Thom (9).
- + *Scopulariopsis* UP 523 (1).
- + *Spicaria violaceae* Abbott (44)*. Even though this fungus was isolated so abundantly from the Transvaal soil, this is the first record of the fungus for South African soil.
- + *Trichoderma koningii* Oud. (1).
- + *Trichoderma pseudokoningii* Rifai agg (1).
- + *Trichoderma viride* (Pers.) Gray (47). As is generally found, this species is one of the most common of soil fungi.
- + *Trichoderma* UP 304 (1).
- + *Verticillium* UP 696 (2).

Dematiaceae

- + *Acremoniella* UP 373 (2).
- + *Alternaria alternata* (Fr.) Keissler (1).
- + *Alternaria tenuissima* (Kunze ex Pers.) Wiltshire (5).
- + *Alternaria* UP 462 (1).
- + *Aurebasidium pullulans* (de Bary) Arnaud (3).
- + *Cadosporium oxysporum* Berk. & Curt. (2).
- + *Cladosporium tenuissimum* Cooke (14). One of the few dematiaceous species that occurred quite frequently.
- + *Cladosporium* UP 428 (1).
- + *Cladosporium* UP 642 (1).
- + *Gonytrichum macrocladum* (Sacc.) Hughes (8)*.
- + *Helminthosporium* UP 662 (1).

Humicola fuscoatra Traaen (16). The most common representative of the Dematiaceae.

Humicola UP 721 (2).

Mammaria UP 835 (1).

Periconia UP 496 (1).

+ *Rhinoctadiella* cf. *elator* Mangelot (1)*. See taxonomic notes below.

Stachybotrys atra Corda (7).

Torula UP 327 (1).

Trichocladium UP 852 (1)*.

Tuberculariaceae

Epicoccum purpurascens Ehrenb. ex Schlecht. (23). Isolated sporadically throughout the survey.

+ *Fusarium equiseti* (Corda) Sacc. (3).

+ *Fusarium graminearum* Schwabe (8).

+ *Fusarium oxysporum* Schlecht (23). By far the most common representative of the family and genus.

+ *Fusarium sambucinum* Fuckel (2).

+ *Fusarium solani* (Mart.) Sacc. (4).

Fusarium UP 326 (1).

Fusarium UP 511 (1).

+ *Fusarium* UP 759 (2).

+ *Fusarium* UP 761 (3).

Volutella ciliata (Alb. & Schw.) Fr. (2)*.

Stilbaceae

Doratomyces stemonites (Pers. ex Fr.) Morton & Smith (12).

+ *Graphium putredinis* (Corda) Hughes (1)*.

Mycelia sterilia

Papulospora UP 517 (1).

Dark sterile mycelium UP 457 (46). Dematioid mycelia were quite common in the open-savannah soil with the type UP 457 being the most common. It could not be induced to sporulate.

TAXONOMIC NOTES ON SOME OF THE ISOLATES

Rhinoctadiella cf. *elator* Mangelot.

Vegetative growth rates on potato-dextrose agar, cornmeal agar and 2% malt-extract agar were 3,5; 1,5 and 1,4 cm in diameter respectively after three weeks of incubation at 25°C. The centre of the colony is raised, woolly and olive-gray in colour. The margin of the colony is flat with appressed mycelium forming concentric rings. Reverse of the colony is dark olive-green. Sporulation is profuse on all these media.

Hyphae very regular, 1–2,6 µm in diameter; no moniliform type seen. Hyphal stands rare. Conidiophores up to 85 µm long, lightly pigmented, forming branches which each terminates in a single, long, denticulate sporogenous tip, 4,5–9,7 µm in length (Figure 1). Proliferation of the sporogenous cell occurs (Figure 2). Sporogenous cells are sympodulae with very pronounced scars in the spore bearing region (Figure 3). Blastospores produced singly from denticles, hyaline, long ellipsoid, 3,8–6,2 × 1,2 × 2,4 µm, smooth.

As pointed out by Schol-Schwarz (1968), the production of phialospores occurs only rarely in some isolates. Hughes (1958) is of the opinion that those types having the *Phialophora* type of spore production should be placed in the genus *Chloridium*. No phialides were produced in the Transvaal isolate. This

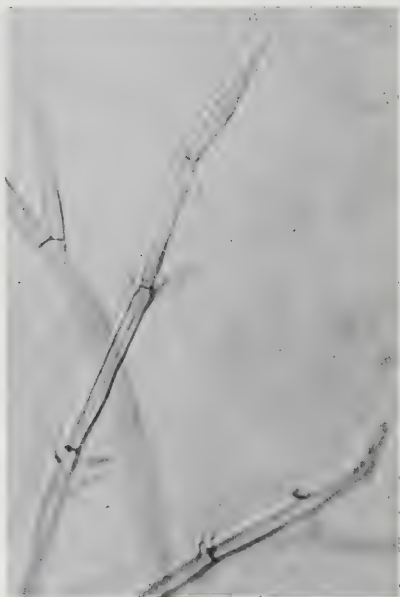


FIG. 1.

Rhinocladiella cf. *elatior*. A denticulate, sporogenous tip of a conidiophore. Note the scars in the spore bearing region. $\times 1000$.

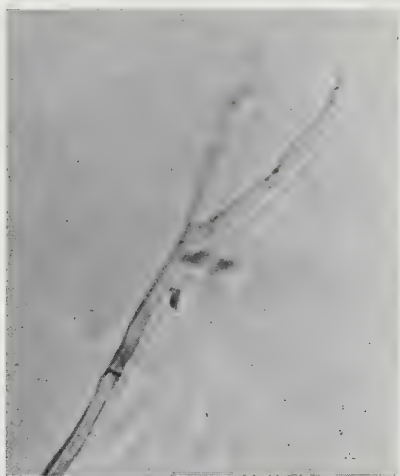


FIG. 2.

R. cf. elatior. A sporogenous cell showing proliferation. $\times 1000$.



FIG. 3.

R. cf. elatior. A scanning electron micrograph of a sporogenous cell showing the very pronounced scars left by conidia. $\times 12000$.

species closely resembles *R. elatior* Mangelot but there are some differences mainly in cultural characteristics. The Transvaal isolate has a slower growth rate and more regular vegetative hyphae and there is no phialospore formation.

SPECIMEN EXAMINED

University of Pretoria 655 (I.M.I. 161, 683) isolated from alkaline soil, Pretoria, November 1971.

This is the first record of the occurrence of the species in South Africa.

Chaetomidium subfimetii Seth.

Colonies on 2% malt-extract agar spreading, light olive-brown. On potato-dextrose agar a very thin, hyaline, spreading mycelium is formed. Perithecia abundant in old cultures, without ostiole or neck, globose, black, attached to the substratum by dark rhizoids (Figure 4). The perithecium is covered by dark brown appendages which are thick walled, roughened, swollen at the base, septate and unbranched with a somewhat hyaline, rounded tip. Asci club-shaped, 8-spored with spores irregularly biserially arranged. Paraphyses rare, seen only in very young ascocarps, filiform, hyaline. Ascospores dark olive-brown, lemon-shaped, $8-11,5 \times 7-10,3 \mu\text{m}$. Apiculated at both ends (Figure 5).



FIG. 4.
Chaetomidium subfimetii. A perithecium, without ostiole, covered by unbranched appendages. S.E.M. $\times 220$.

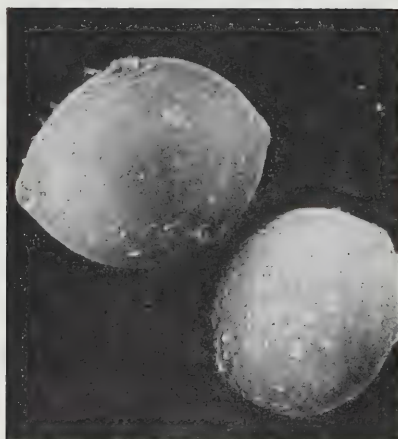


FIG. 5.
C. subfimetii. Very characteristic lemon-shaped ascospores S.E.M. $\times 4\,500$.

SPECIMEN EXAMINED

University of Pretoria 346 (I.M.I. 162, 626) isolated from alkaline soil, Pretoria, March 1972.

This isolate resembles most closely *C. subfimetii* as described by Seth (1967) but differs from it mainly in the somewhat larger ascospore size. The ascospores are, however, not large enough to warrant the inclusion of this fungus in *C. fimeti* Fuckel.

This is the first record of the occurrence of this genus in South Africa.

Auxarthron umbrinum (Boudier) Orr & Plunkett

Colonies on potato-carrot agar slow growing, granular, at first white, later turning yellowish brown. Underside of colony yellow to orange. A wine-red pigment diffuses into the medium. Ascocarps embedded in superficial mycelium, spherical, reddish-brown, diameter excluding appendages 150–460 μm . Peridial hyphae yellow, 2,2–4,4 μm in diameter, asperate (Figure 6) thick-walled, anastomosed dichotomously to form a reticulate peridium; septate with swollen knuckle joints measuring 4,4–6,1 μm in diameter, ending in numerous short, roughened spinelike appendages with rounded or acute apices (Figure 7) or aseptate or with 1 or 2 septa, 3,3–69,0 μm in length. Few elongate appendages, $\pm$ smooth, sometimes branched, thick-walled, yellow-brown becoming discoloured towards an acute apex, aseptate except for one or two knuckle-joints near base, usually straight or apex slightly hooked, diameter at base 4,0–5,0 μm and up to 860 μm in length.

Asci hyaline, subglobose to ovoid, thin-walled, evanescent, 8 spored, 5,5–6,5 $\times$ 6,5–7,6 μm . Ascospores almost hyaline, smooth at all stages with a reticulate appearance, oblate, i.e. globose to subglobose from above and compressed in side view, 2,1–2,8 μm , average 2,5 μm in diameter (Figure 8). Asexual stage not observed.



FIG. 6.
Auxarthron umbrinum. Portion of a peridial hypha showing the asperate nature of its surface. S.E.M. $\times$ 12 500.

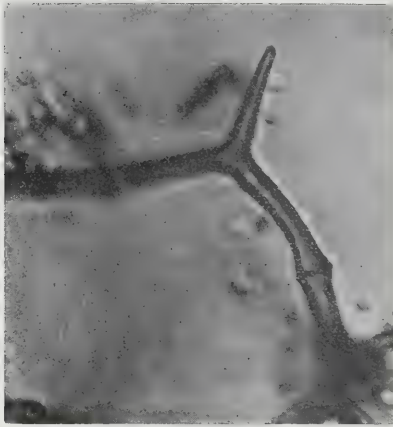


FIG. 7.

A. umbrinum. A spinelike appendage in the region of a swollen knuckle joint of the peridial hyphae. $\times 1\ 000$.

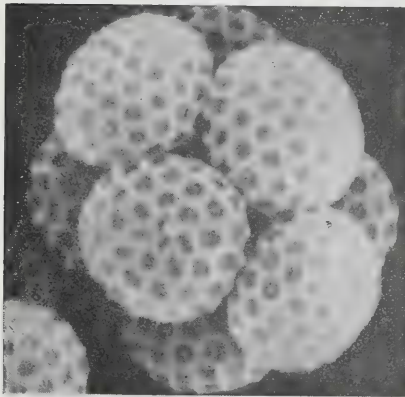


FIG. 8.

A. umbrinum. A group of eight ascospores showing the arrangement in an ascus. The spores are smooth and reticulate. S.E.M. $\times 10\ 000$.

SPECIMEN EXAMINED

University of Pretoria 433 (I.M.I. 164, 235). Isolated from alkaline soil, Pretoria, August 1971.

This isolate is most similar to *A. umbrinum* as described by Orr, Kuehn and Plunkett (1963) and also to an isolate of the same species described by Papen-

dorf (Marasas *et al.*, 1966) from litter of *Acacia karroo* in Transvaal. The isolate differs from previous descriptions of *A. umbrinum* mainly in the ascospores being at all times smooth and reticulate, the maximum diameter of the ascocarp and of the ascospores, and the infrequent branching of shorter appendages. An interesting characteristic which has not previously been reported for these fungi is the presence of branched, elongate appendages.

Apinis (1964), in his revision of the British Gymnoascaceae, placed this species in the genus *Gymnoascus* (subgenus *Auxarthron*). Most modern authors retain the genus name *Auxarthron* (Von Arx, 1970; Ainsworth, Sparrow & Sussman, 1973).

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REFERENCES

- ACOCKS, J. P. H., 1963. Veld types of South Africa. *Mem. bot. Surv. S.Afr.* **28**: 1-192.
- AINSWORTH, G. C., SPARROW, F. K. and SUSSMAN, A. S., (eds.), 1973. *The Fungi, an advanced treatise*. Vol. VI A. New York: Academic Press.
- APINIS, A. E., 1964. Revision of British Gymnoascaceae. *Mycol. Pap.* **96**: 56.
- EICKER, A., 1969. Microfungi from surface soil of forest communities in Zululand. *Trans. Br. mycol. Soc.* **53**: 381-392.
- EICKER, A., 1970 a. Vertical distribution of fungi in Zululand soils. *Trans. Br. mycol. Soc.* **55**: 45-57.
- EICKER, A., 1970 b. Ecological observations on soil fungi. *S.Afr. J. Sci.* **66**: 327-334.
- GAMS, W. and DOMSCH, K. H., 1967. Beiträge zur Anwendung der Bodenwaschtechnik für die Isolierung von Bodenpilzen. *Arch. Mikrobiol.* **58**: 134-144.
- HUGHES, 1958. Revisions Hyphomycetum aliquot cum appendice de nominibus rejiciendis. *Can. J. Bot.* **36**: 727-836.
- LEACH, C. M., 1962. Sporulation of diverse species of fungi under near-ultraviolet radiation. *Can. J. Bot.* **40**: 151-161.
- MARASAS, W. F. O., VAN DER WESTHUIZEN, G. C. A., VAN WARMELO, K. T. and PAPENDORF, M. C., 1966. New and interesting records of South African fungi. Part V. *Bothalia* **9** (1): 229-243.
- MARTIN, J. P., 1950. Use of acid, Rose Bengal and streptomycin in the plate method for estimating soil fungi. *Soil Sci.* **69**: 215-232.
- MENZIES, J. D., 1957. A dipper technique for serial dilutions of soil for microbial analysis. *Soil Sci. (Amer. Proc.)* **21**: 660.
- ORR, G. F., KUEHN, H. H. and PLUNKETT, C. A., 1963. A new genus of the Cymnoascaceae with swollen peridial septa. *Can. J. Bot.* **41**: 1 439-1 456.
- PAPENDORF, M. C., 1967. Two new genera of soil fungi from South Africa. *Trans. Br. mycol. Soc.* **50**: 69-75.
- PAPENDORFF, M. C., 1969. *Leptodiscella africana* gen. et comb. nov. *Trans. Br. mycol. Soc.* **53**: 145-147.
- PAPENDORF, M. C. and DU TOIT, J. D., 1967. *Melanophoma*, a new genus of the Sphaeropsidales. *Trans. Br. mycol. Soc.* **50**: 503-506.
- PAPENDORF, M. C. and VON ARX, J. A., 1966. *Herpotrichia striatispora*, a new Ascomycete from South Africa. *Nova Hedwigia* **12**: 395-398.

- SCHOL-SCHWARZ, M. E., 1968. *Rhinocladiella*, its synonym *Fonsecaea* and its relation to *Phialophora*. *Antonie van Leeuwenhoek* **34**: 119-152.
- SETH, H. K., 1967. *Chaetomidium subfimetii* sp. nov. from Wales. *Trans. Br. mycol. Soc.* **50** (1): 45-47.
- VON ARX, J. A., 1970. *The genera of fungi sporulating in pure culture*. Lehre: J. Cramer.
- WAKSMAN, S. A. and FRED, B., 1922. A tentative outline of the plate method for determining the number of micro-organisms in the soil. *Soil Sci.* **14**: 27-28.
- WARCUP, J. H., 1950. The soil-plate method for isolation of fungi from soil. *Nature, Lond.* **166**: 117-118.